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GEOPHYSICAL VARIABLES AND BEHAVIOR: XCIX. REDUCTIONS IN NUMBERS OF NEURONS WITHIN THE PARASOLITARY NUCLEUS IN RATS EXPOSED PERINATALLY TO A MAGNETIC PATTERN DESIGNED TO IMITATE GEOMAGNETIC CONTINUOUS PULSATIONS: IMPLICATIONS FOR SUDDEN INFANT DEATH[1,2]

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Summary.—Correlational analyses have shown a moderate strength association between the occurrence of continuous pulsations, a type of geomagnetic activity within the 0.2-Hz to 5-Hz range, and the occurrence of Sudden Infant Deaths. In the present study, rats were exposed continuously from two days before birth to seven days after birth to 0.5-Hz pulsed-square wave magnetic fields whose intensities were within either the nanoTesla or microTesla range. The magnetic fields were generated in either an east-west (E-W) or north-south (N-S) direction. At 21 days of age, the area of the parasolitary nucleus (but not the solitary nucleus) was significantly smaller, and the numbers of neurons were significantly less in rats that had been exposed to the nanoT fields generated in the east-west direction or to the microTesla fields generated within either E-W or N-S direction relative to those exposed to the N-S nanoTesla fields. These results suggest nanoTesla magnetic fields, when applied in a specific direction, might interact with the local geomagnetic field to affect cell migration in structures within the brain stem that modulate vestibular-related arousal and respiratory or cardiovascular stability.

The occurrence of Sudden Infant Death has been correlated with various increments of geomagnetic activity during the months in which the deaths occur. More specifically, O'Connor and Persinger (1997) found a strong correlation between the incidence of Sudden Infant Deaths in Canada and specific bands of intensity of geomagnetic activity. Their subsequent analyses (O'Connor & Persinger, 1999) suggested a role of protracted periods of weak (< 1 nT) symmetrical rhythmic activity, continuous geomagnetic pulsations (pc1), in the association between global geomagnetic activity and Sudden Infant Death.

Although there are many hypotheses concerning the cause of sudden death in infants, anomalies within the neuronal arrangement or electrical activity of specific structures in the brain stem have been considered the

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most common predisposing factor. Transient anomalous dysfunctions that lead to recurrent intermittent episodes of hypoxia may contribute to sudden death. Waters, Meehan, Huang, Gravel, Michaud, and Cote (1999) found that most of the infants who died suddenly, compared to the reference group, displayed apoptosis of neurons within the brain stem. The resultant reduction of cell numbers would be compatible with the magnitude of the marginally significant cytometric changes in neuronal soma, specifically within medullary structures that modulate heart rate and respiration, in the brains of infants who died suddenly (Oehmichen, Linke, Zilles, & Saternus, 1989).

The present experiment was designed to imitate a temporal pattern of specific geomagnetic conditions, particularly the simulation of repeated rhythmic shapes of magnetic fields (similar to pc1 pulsations), that might contribute to Sudden Infant Death. Because the incidence of Sudden Infant Death is relatively low, it was suspected that it would be produced by a series of interacting variables. One of these would involve the activities of the medullary reticular nuclei that affect arousal. Recently, Barmack and Yakhnitsa (2000) showed that the parasolitary nucleus is the source of GABAnergic projections to two regions: the inferior olive and the nucleus reticularis gigantocellularis. They are involved with the level of physiological arousal.

If the occurrence of a specific geomagnetic pattern during a critical period of neurodevelopment affected cell numbers in the parasolitary nucleus, then subsequent exposure to these fields might produce an electrical anomaly that would produce cessation of respiration and heart beats (Eckert, 1992). For comparison and to help control for the role of "nonspecific effects," the solitary nucleus was selected for examination. It is adjacent to the parasolitary nucleus. Unlike the parasolitary nucleus, the solitary nucleus receives sensory fibers from the facial, glossopharyngeal, and vagus nerves. Whereas the cells within the rostral third of this nucleus are associated with gustation, those within the posterior two-thirds are involved with visceral afferents.

Method

In two separate blocks, a total of 16 pregnant, approximately 90-day-old Wistar albino rats housed singly in standard plastic cages (with aluminum tops containing food and a water bottle) were exposed to magnetic fields produced by one of two 112-cm by 125-cm aluminium racks that were separated by at least 2 m and arranged either in an east-west or north-south direction. Each rack was wrapped around its perimeter with 72 turns of 30-gauge (AWG) copper wire to form a band 13 cm wide.

In different blocks of the experiments, one coil served as the active coil while the other served as the reference coil. As a result, there were four lit-

ters for each of the four conditions. When a coil was activated a 0.5-Hz pulsed-square wave magnetic field was generated continuously through the coil and the cages by a custom-made device. When the current was delivered through the coil the magnetic field was generated in the perpendicular direction within the horizontal plane. The resulting fields, as measured by a MEDA FM-300 magnetometer, were a continuous rhythmic series of signals. The total duration of each complex square wave that composed the continuous rhythmic signals was about 600 msec. Its onset was associated with a transient spike with a rise time of between 1.5 and 3.5 microsec. that was about four times the amplitude of the major plateau. The offset was associated with a similar spike with opposite polarity with comparable amplitude.

The amplitudes of the continuously presented 0.5-Hz waves were changed every 30 min. These protracted changes in amplitude occurred in 4-hr. cycles with six cycles per day (McKay, Koren, & Persinger, 2003). The duration of 4 hr. was selected given its similarity to natural pulsations. The maximum intensities of the amplitude modulations according to the magnetometer (placed within the same plastic cage and lid involved with housing the mothers) during each 30-min. period were 0, 870 nT, 1040 nT (1.04 microT), 1300 nT (1.30 microT), 1770 nT (1.77 microT), 1330 nT, 1040 nT, and 870 nT within the active coil. Given induction effects, the maximum field strengths in the inactive coil per successive 30-min. period were 0, 4 nT, 8 nT, 11 nT, 13 nT, 11 nT, 8 nT, and 4 nT. These changes were superimposed upon the ambient geomagnetic activity (see McKay, *et al.*, 2003 for sample figures). The rats were exposed from two days before birth to seven days after birth. This period was selected because it involved the perinatal period shown to be most sensitive to the effects of magnetic fields (Persinger, Lafreniere, & Ossenkopp, 1974). The litters were not culled so that potential postnatal deaths could be detected. Except to discern whether the pups were alive once per day, they were not touched.

At 21 days of age, one male and one female rat from each litter were randomly selected and decapitated. The 32 brains were fixed in ethanol-formalin acetic acid, processed, embedded in paraffin, and sectioned at 10 micrometers. The required sections were stained with toluidine blue O. The numbers of neurons and glial cells within the solitary nucleus and parasolitary nucleus were counted in a 6×6 grid at 400 X. The sections included the parasolitary nucleus in 28 of the rats and the solitary nucleus in 31 of the rats. However, the four treatments were more or less equally represented. Three-way analyses of variance as a function of sex, field (activated or reference), and orientation of the applied field (E–W vs N–S) were completed for the numbers of neurons, glial cells (oligodendroglia and astroglia), and neuronal/glial ratios by SPSS software on a VAX 2000 computer. Significance by Tukey *post hoc* tests was set at $p < .05$.

Results

The means and standard deviations for numbers of neurons and glial cells, neuronal/glia ratios, and areas for the two structures are shown in Table 1. There were significantly ($F_{1,19}=4.79$, $p<.05$; $\eta^2=.36$) fewer neurons within the parasolitary nuclei of rats that had been exposed to the activated field compared to the reference field. However, this main effect was confounded by the statistically significant interaction between the orientation of the applied field and its intensity ($F_{1,19}=9.28$, $p<.01$). *Post hoc* analysis indicated that the rats exposed to the microT fields showed reduced numbers of neurons, regardless of field orientation. Those exposed to the nanoT field generated in the E–W direction also showed reduced numbers of neurons compared to those exposed to the nanoT field generated in the N–S direction. The areas of the parasolitary nuclei of the rats exposed to the microT field, regardless of coil orientation, were significantly smaller ($F_{1,19}=4.25$, $p<.05$; $\eta^2=.37$) relative to those exposed to the nanoT intensities.

TABLE 1

Means and Standard Deviations For Numbers of Neurons, Glial Cells, Neuronal/Glial Ratios and Area (um^2) of the Structure Per Section Within the Parasolitary and Solitary Nuclei in Rats Perinatally Exposed to Various Simulated "Geomagnetic" Conditions

	nanoTesla Field				microTesla Field			
	Direction of Applied Field							
	N–S		E–W		N–S		E–W	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Parasolitary Nucleus								
Area (um^2)	34720[a]	15190	26474	6553	19288[b]	5520	24159	7194
Neurons	152[a]	66	88[b]	11	75[b]	30	93[b]	20
Glial cells	47	28	44	19	40	19	42	17
Neuron/Glial cells	3.5	.8	2.3	.9	2.2	1.0	2.4	.8
Solitary Nucleus								
Neurons	4413	196	3462	620	3897	1019	3921	564
Glial cells	67[a]	14	88	15	104[b]	13	91	16
Neuron/Glial cells	2.6[a]	.3	1.9	.6	1.5[b]	.4	2.1[a]	.4

Note.—Fields are applied in a north–south (N–S) or east–west (E–W) direction.
[a vs b] $p<.05$.

Except for the significantly ($F_{1,22}=7.26$, $p<.01$; $\eta^2=.43$) fewer numbers of neurons within the solitary nucleus for the male brains ($M=157$ neurons, $SD=30$) compared to those for the female brains ($M=180$ neurons, $SD=24$), there were no statistically significant treatment effects or interactions between treatments and sex. However, there were significantly more glial cells ($F_{1,22}=4.15$, $p<.05$; $\eta^2=.32$) in the solitary nuclei of rats that had been exposed to the microT fields compared to the nanoT field. The interaction between the orientation and the activation of the fields was significant ($F_{1,22}=$

7.49, $p = .01$) and, due to the fact that the rats exposed to the microT fields generated in the N–S direction, had more glial cells than those exposed to the nanoT field while this discrepancy was not present for those exposed to the field generated in the E–W direction.

By far the most powerful treatment effect was for the ratios of neurons to glial cells within the solitary nucleus. Male rats displayed significantly ($F_{1,22} = 12.30$, $p < .001$; $\eta^2 = .43$) lower ratios than did the females. There was a significant interaction ($F_{1,22} = 15.63$, $p < .001$) between intensity of the field and its orientation. *Post hoc* analysis indicated that rats exposed to the microT field generated in the north–south direction showed lower ratios than those exposed to the nanoT field generated in the north–south direction while those exposed to the microT or nanoT fields generated in the east-west direction had comparable values.

Discussion

The results of this study suggest that the geomagnetic orientation of the source generating complex magnetic fields designed to simulate geomagnetic pulsations was a significant variable. The rats that had been exposed to the nanoTesla strength magnetic fields generated in an east–west direction displayed fewer cells within the parasolitary nucleus compared to the rats that had received the same-strength field generated in a north–south direction. Rats that had been exposed to the field strengths one-thousand times stronger than the nanoTesla ranges displayed comparable reduced numbers of cells, regardless of the fields' orientations.

The results of the study do not indicate whether the source of the diminished numbers of neurons was necrosis or apoptosis. Significant neuronal apoptosis was found in the brain stem for 96% of infants who had died suddenly (Waters, *et al.*, 1999). The distribution of apoptosis was mostly in dorsal nuclei, including the spinal trigeminal tract and the vestibular nuclei. These structures are involved with sensation to the face and position of the head. Waters, *et al.* (1999) suggested that enhanced neuronal death by apoptosis and the involvement of these specific brain stem nuclei might be linked to the role of prone sleeping as a significant risk factor for Sudden Infant Death.

It was assumed that the values for the numbers of neurons within a cross-section of the parasolitary nucleus and its cross-sectional area in the rats that had been exposed perinatally to the nanoTesla 0.5-Hz fields generated in a north–south direction are typical of normal estimates. Consequently, the lower numbers of neurons seen in rats exposed to the nanoTesla fields generated in an east–west direction would reflect an effect from this treatment. If this assumption is valid then geomagnetic pulsations (pc1) or less symmetrical temporal variations of correlative geomagnetic activity gen-

erated along this spatial dimension could be important for affecting the development of neuronal conditions that might increase the organism's later sensitivity to specific patterns of weak magnetic fields.

Our daily magnetometer recordings (24-sec. samples) have clearly shown that on some days only the north–south, east–west, or vertical component may be the most affected component of the earth's magnetic field during a geomagnetic storm. Global values, such as the average antipodal index (aa), reflect the most "disturbed" magnetometric components. As a result days with similar quantitative values may have been dominated by qualitatively different types of disturbances. If the spatial vector of the geomagnetic disturbance is a critical variable, then gross correlations between numbers of Sudden Infant Deaths and aa values might underestimate the strength of the true association.

Cells within the parasolitary nucleus are the primary source of the inhibitor neurotransmitter GABA to portions of the inferior olive and giant reticular nucleus. Cells from components of the inferior olive project to the contralateral cerebellar uvula-nodulus (Barmack, 1996) that project back to the parasolitary nucleus. The average discharge rate for these cells in response to sinusoidal stimulation between 0.2 Hz and .4 Hz was about 10 Hz (Barmack & Yakhnitsa, 2000). The activities of these cells were also modulated by vestibular stimulation in either the pitch or roll planes (dynamic roll-tilt along the longitudinal plane) but not the horizontal plane. If during development these cells were affected by pc1-like geomagnetic patterns, then head movements within a particular plane could significantly affect the stability and frequency of the discharge rates of aggregates of these neurons.

Such responses to stimuli with specific orientation may explain the relationship between the developmental window associated with Sudden Infant Death and periods when head movements, particularly when the infant is placed on its stomach, are prominent (Stulbach, 2003). It is suggested that appropriate synchronization by periodic geomagnetic oscillations within the pc1 range (around 0.5 Hz) when presented with the optimal ratio of time-varying-to-static intensities produces a resonance within the parasolitary circuit. Because the parasolitary nucleus lacks a commissural projection, mutual or reciprocal inhibition does not occur, and the inhibitory discharges become markedly synchronized. A reduction in the number of neurons as seen in this study may encourage this synchronization if the neurons that have died or failed to develop determined primarily inhibitory intranuclear functions.

It may be of interest that the locus ceruleus, which processes sensory input and regulates the forebrain's functional state, exhibits intrinsic oscillations with periods in the .02-Hz to .5-Hz range (Filippov, Gladyshev, &

Williams, 2002). The presence of these "infraslow potentials" as well as their amplitudes can be exacerbated by the stress of physical restraint. In infants under six months of age, lying on the ventral surface (face down) may simulate this stress. Developmental abnormalities within the medullary reticular formation have been strongly implicated in at least a subset of Sudden Infant Deaths (Kinney, Filiano, & White, 2001). If our hypothesis is correct, the paucity of neurons within at least the parasolitary nucleus may reflect a network of developmental deficiencies that, given the specific combination of stimuli, result in sudden death.

That the results of our study were nonspecific artifacts was not likely in light of the absence of significant group differences for the numbers of neurons within the nearby solitary nucleus. However, there were significantly greater numbers of glial cells within the solitary nucleus of the rats that had been exposed to the east–west generated nanoTesla fields, compared to the rats that had been exposed to the north–south generated fields. If we assume that increased numbers of glial cells suggest reactive gliosis from an earlier period, then their evaluation in conjunction with the reduction of neurons might reflect the consequences of early deafferentiation. The present model would predict that infants who die suddenly must have been exposed several times to similar stimuli during their development to produce the deficit that would allow the precipitating stimulus within the geomagnetic field to produce sudden death.

This model would be similar to the predictions of Eckert (1992) who hypothesized two elements were required, a selection factor and a trigger factor, for the occurrence of Sudden Infant Death. The first involved disturbed geomagnetic fields within the residence or surroundings of a pregnant woman that interrupted the normal development of the central organ that controls respiration in the fetus. Were this infant then exposed to an electromagnetic pulsation similar to its own breathing frequency, but inverted in phase, value, or form (the trigger factor), the impulses from the "respiratory control organ" to the breathing organs would be disturbed. This would have a fatal effect.

There is one final implication of this reasoning. If vulnerable organisms do not die as infants, they may be vulnerable, perhaps as adults, to other physiological transients such as cardiac arrhythmias. Persinger and O'Connor (2001) found increased incidences of cardiac arrhythmias in adults and sudden deaths in infants occurred on the same days. In our first series of experiments of reintroducing adult rats to the 0.5-Hz, 5-nT to 10-nT field in which they were born, there was a sudden cardiac death in a healthy 4-mo.-old adult male rat within one hour of an abrupt and persistent decrease in the intensity of the north–south component of the geomagnetic field within the coils.

Although the vertical component remained relatively stable during this event, there was a decrease from 4,250 nT to 4,158 nT (102 nT) within the X-component (N–S direction) of the earth's magnetic field over a period of 1.8 hr. Superimposed on this gradual decline was a rapid (within a few minutes) decrease of 45 nT followed 28 min. later after no change by another rapid decrease of 36 nT. There were concomitant rapid increases of about 10 to 20 nT in the Y-component (E–W direction) during these periods. This same event in more northern latitudes, for example Finland (Sodankyla Geophysical Laboratory), was associated with a comparable temporal structure but a greater decrease of more than 500 nT in the X-component.

The rat had been placed in the exact cage position (and static geomagnetic configuration) and orientation (E–W) of the experimental magnetic field in which he had been born four months previously. There has never been a sudden death in this age range of normal rats in the population of approximately 4,000 rats we have studied over the last 20 years. However, we (Persinger, McKay, O'Donovan, & Koren, in revision) have found that nocturnal exposures for only 6 min. once per hour to experimental fields whose intensities decreased by about 50 nT in the north–south direction resulted in increased sudden death in male rats within 24 hr. after epileptic seizures had been induced.

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